August 14, 1973

Dr. Paul Hough
Cold Spring Habbor Laboratory of
Quantitative Biology
Cold Spring Harbor
Long Island, New York

Dear Dr. Hough:

My colleague Mike Bishop recently returned from CSH with a report (via Nancy Hopkins) that you have been extremely successful in enucleating relatively large numbers of cells and that you might be interested in a collaborative venture dealing with the mechanism of proviral DNA synthesis by Rous sarcoma virus. As the accompanying preprints indicates, we have been studying the synthesis of RSV-specific DNA in both permissive (duck) and non-permissive (mammalian, mainly murine) hosts.

In duck cells we can detect viral DNA synthesis as early as 3 hours after infection (by subgroup C viruses), and at this point all of the DNA is in the cytoplasm as determined by assay of conventionally-fractionated cytoplasm and nucleic. (Cells are washed, swollen in 0.1xRSB, dounced in the presence of NP40 and DOC, and nuclei spun out at low speed.) The amount of DNA increases in the whole cell up to 16-24 hours, but by 16 hours it is detectable only in the nuclear fraction. Integration (as measured by the network test) begins by 9 hours or so, and is completed within 24-36 hours. Preliminary sizing of either cytoplasmic or unintegrated DNA suggests that the <u>in vivo</u> product is about the size of a copy of a subunit of RNA, I.e., a double stranded molecule of about 6×10^6. Early in infection, there appears to be both single and double stranded DNA in the cytoplasm. We do not yet know if this DNA is infectious, but we are now testing this. We are also in the process of looking at the susceptibility of unintegrated (and integrated) proviral DNA to restriction endonucleases.

Viral DNA synthesis by RSV after mass infection of mammalian cells (we use Todaro's A31 clone of BALB/c 3T3 cells) is observable only with virus previously rescued(by cocultivation with a permissive host) from RSV-transformed mouse or rat cells. Presumably this phenomenon is related to the increased efficiency of transformation (from very poor to poor---i.e., from 10^{-5} to 10^{-4} or 10^{-3} in comparison with focus formation in chick cells) by rescued virus, as noted by Altaner and Temin. DNA synthesis (to the extent of 1-2 copies/cell) is detectable by 12 hours (and perhaps earlier---we haven't looked); integration begins after 12 hours and is complete within 60 hours; the DNA is stably inherited for at least several generations. It is overwhelmingly likely that the vast majority of cells are infected (as measured by viral DNA synthesis) but not transformed (as measured by ability to clone in soft agar); we are in the process of testing this more stringently by examining clones of infected cells known not to grow in soft agar. Surprisingly, 60 hours after this largely abortive infection there is considerable transcription of viral RNA (as much

as 10 times more RNA than we see in a clone of transformed mouse cells and it is copied from at least 80% of the genome as measured by a competition hybridization assay).

I hope you will agree with me that a fusion of our technologies might allow us to ask some very interesting questions about the synthesis and characteristics of proviral DNA in these two types of host cell. To give you some idea of the logistics that might be required, we currently use about $5\text{-}10 \times 10^7$ cells to measure synthesis of 1-4 copies of viral DNA per cell. It is conceivable that higher multiplicities of infection and prolonged incubation might result in more viral DNA synthesis per cell with enucleated cells. How many cells can you process at once (the report I heard was 10^7)? What varieties of cells have you tried? How long do your cells remain healthy after enucleation? What percent remain nucleated (rumor has it this is less than 1%)?

If you are interested in pursuing some of these matters, we could proceed in a number of ways: I could simply send you the necessary viruses and receive the partially extracted cells back from you; if convenient for you, you would be welcome to come out and work in our lab for a couple of weeks to get this effort started; conceivably, I could come there for a short period, equipped with viruses, but a soon-to-be-delivered first baby makes this prospect unlikely in the near future. Please write or call me here (415-666-2824) when you've had a chance to digest this, and we can consider the possibilities further.

Yours,

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Department of Microbiology

Encl: MEV: Lepetit
Squaw Valley
PNAS - Integration paper
J. Mol. Biol. reprint